Scientific Interaction among National Interagency Confederation for Biological Research (NICBR) Partners

Protective Vaccination of Primates against Ebola Virus Infection

Ebola and Marburg viruses are significant bioterrorist threats. USAMRIID and NIAID are collaborating to develop safe and effective vaccines to protect both military and civilian populations against aerosol attack.

Initial studies of the effectiveness of vaccination with Adenovirus vectors (ADV) expressing either the glycoprotein (GP) or nucleoprotein (NP) gene of Ebola virus were studied using either a prime/boost or a single-dose immunization regimen:

- Monkeys were vaccinated with either ADV-GP and ADV-N, boosted after 9 weeks, and then challenged on week 10 with either low or high doses of virulent Ebola (Zaire strain). A combination of ADV-GP plus ADV-NP protected primates against a robust, Ebola-Zaire challenge in this prime/boost regimen.
- To test an accelerated vaccine regimen, monkeys were immunized with a single vaccine dose and challenged 4 weeks later. Protection was achieved within 28 days of a single ADV-GP/NP shot.

Subsequent studies published in 2006 documented that ADV-GP alone (without ADV-N) was effective in a single dose of 10^10, although 10-fold lower doses were ineffective.

Cross-challenge studies indicated that GP from both Ebola-Zaire and Ebola-Sudan would be required in a bivalent vaccine to afford protection against both strains.

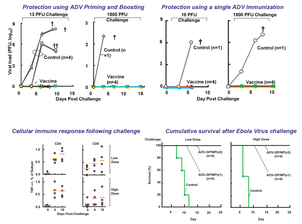
Next steps:

- Developing vaccines providing cross protection against Ebola-Sudan strains
- Testing against aerosol challenge
- Developing analogous ADV vectors for Marburg viruses
- Manufacture of clinical grade vaccine for human use
- Testing DNA-prime adenovirus boost strategies to circumvent pre-existing adenovirus-5 immunity and enhance durability of the protective response.

Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates. Nature 424:681-684 Aug 2003.

Nancy J. Sullivan, Thomas W. Geisbert, Joan B. Geisbert, Ling Xu, Zhi-yong Yang, Mario Roederer, Richard A. Koup, Peter B. Jahrling & Gary J. Nabel Immune Protection of Nonhuman Primates Against Ebola Virus with Single Low-Dose Adenovirus Vectors Encoding Modified GPs

Nancy J. Sullivan, Thomas W. Geisbert, Joan B. Geisbert, Devon J. Shedlock, Ling Xu, Laurie Lamoreaux, Jerome H. H. V. Custers, Paul M. Popernack, Zhi-Yong Yang, Maria Grazia Pau, Mario Roederer, Richard A. Koup, Jaap Goudsmit, Peter B. Jahrling, Gary J. Nabel , PLOS Medicine 3: June 2006 (on line).

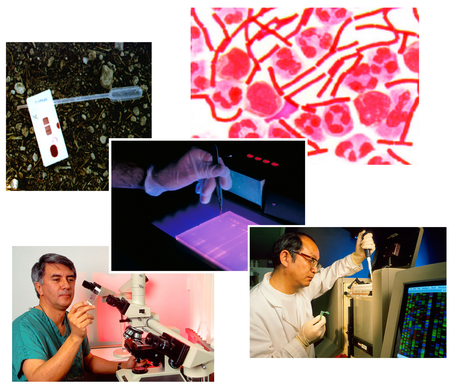


National Bioforensics Analysis Center

The 2001 anthrax attacks in the United States demonstrated the need for a dedicated biocontainment facility to coordinate and conduct bioforensic analysis, as well as perform traditional forensic analyses of biohazardous evidence. In April 2004, the National Bioforensics Analysis Center (NBFAC) was created by Homeland Security Presidential Directive 10 within the National Biodefense Analysis and Countermeasures Center (NBACC) to be

"…the lead Federal facility to conduct and facilitate technical forensic analysis and interpretation of materials recovered following a biological attack in support of the appropriate lead Federal agency."

To accomplish this mission, the Department of Homeland Security (DHS) and Department of Defense (DoD) entered into an agreement in which the United States Army Medical Research Institute for Infectious Diseases (USAMRIID) provides BSL3 biocontainment space for NBFAC at USAMRIID and contract staff to support the NBFAC mission. This DoD and DHS collaboration established the first dedicated bioforensic containment laboratory within the United States. Commissioned in May of 2004, the NBFAC Interim "Hub" laboratory located at USAMRIID immediately began supporting the FBI and other federal government customers.

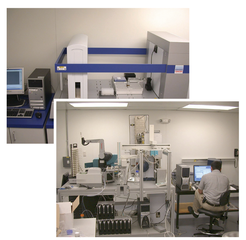


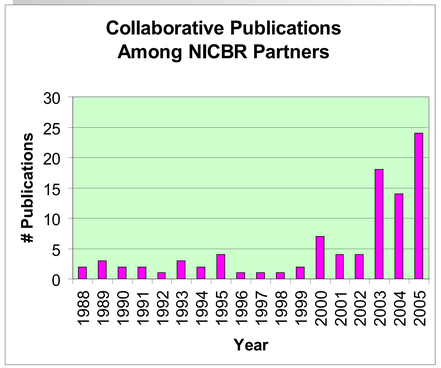
Rapid Pathogen Detection Using TIGER Biosensor System

Scientists from the USDA-ARS Foreign Disease-Weed Science Research Unit and USAMRIID are partnering with Ibis Therapeutics on a project utilizing TIGER (Triangulation Identification for Genetic Evaluation of Risks) technology for rapid identification of microbial pathogens. The TIGER system at Fort Detrick became fully operational in January 2006. TIGER represents "a strategy for the detection and characterization of microorganisms associated with a potential biological warfare attack or a natural outbreak of an emerging infectious disease." (Hofstadler et al. 2005).

The TIGER system applies polymerase chain reaction and mass spectrometry to rapidly identify previously uncharacterized pathogens, requiring no previous knowledge of the pathogen genotype. Working on a grant from the USDA, CSREES Plant Biosecurity program, Drs. William Schneider and Elena Postnikova (ARS-FDWSRU) and Chris Whitehouse (USAMRIID, Diagnostic Systems Division) are currently designing and testing oligonucleotide primer sets for identification of high-priority pathogens on the TIGER platform located at USAMRIID.

Hofstadler et al. 2005. TIGER: the universal biosensor. International Journal of Mass Spectrometry 242:23-41, 2005.





Rapid Response to SARS Pandemic

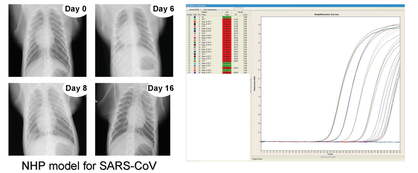
CDC, NIAID, USAMRIID, and Intermune, Inc. worked together during the spring and summer of 2003 to develop rapid and effective responses to the SARS pandemic, a highly lethal lower respiratory disease in humans caused by a newly emergent coronavirus. The collaboration took advantage of the strengths of all the organizations, and led to development of a full array of countermeasures and research tools to support ongoing efforts and combat future outbreaks. Major achievements include:

- Diagnostics: rapid nucleic acid detection assays and antibody screening reagents were developed which should allow early detection and thus more rapid containment of subsequent outbreaks
- Animal Models: Isolates of SARS were used to develop an aerosol model for SARS in non-human primates for preclinical testing of potential SARS countermeasures
- Therapeutics: in vitro screening of several hundred thousand compounds for anti-SARS activity led to identification of several active compounds that later entered clinical trials.

Lawler JV, Endy TP, Hensley LE, Garrison A, Fritz EA, Lesar M, Baric RS, Kulesh DA, Norwood DA, Wasieloski LP, Ulrich MP, Slezak TR, Vitalis E, Huggins JW, Jahrling PB, Paragas J. Cynomolgus Macaque as an animal model for severe acute respiratory syndrome. PLoS Med. Apr 18;3(5):e149, 2006.

Paragas J, Blatt LM, Hartmann C, Huggins JW, Endy TP. Interferon alfacon1 is an inhibitor of SARS-corona virus in cell-based models. Antiviral Res. Jun;66(2-3):99-102, 2005. Epub 2005 Feb 15.

Loutfy MR, Blatt LM, Siminovitch KA, Ward S, Wolff B, Lho H, Pham DH, Deif H, LaMere EA, Chang M, Kain KC, Farcas GA, Ferguson P, Latchford M, Levy G, Dennis JW, Lai EK, Fish EN. Interferon alfacon-1 plus corticosteroids in severe acute respiratory syndrome: a preliminary study. JAMA. Dec 24;290(24):3222-8, 2003.



NHP model for SARS-CoV

Therapeutic Countermeasures for Bioterrorism

NCI and USAMRIID are working together to develop effective therapeutic countermeasures against Class A biothreat agents. The collaboration brings together USAMRIID's extensive select agent expertise and NCI's unparalleled drug discovery and development capability. Cutting-edge informatics and molecular modeling technologies coupled with state-of-the-art biological testing have resulted in the discovery of diverse sets of new chemical scaffolds with proven inhibition against critical targets from anthrax, botulinum as well as filoviruses such as Ebola and Marburg. The success of this collaboration has attracted a number of academic partners, including Harvard University, Howard Hughes Medical Institute, Scripps, Burnham Institute, University of Nebraska, University of Pittsburgh, University of Belgrade, and the European Molecular Biology Laboratory. Recent accomplishments include:

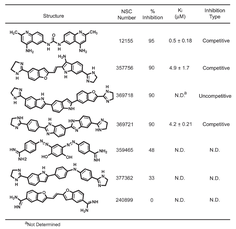
- Discovery of a new dual-function inhibitor that is both antibiotic and inhibits the Anthrax Lethal Factor toxin. This inhibitor is active against the ciprofloxacin-resistant strain of anthrax, with a potency that is twice that of ciprofloxacin against anthrax itself.
- Identification of potent inhibitors for Botulinum Neurotoxin A. One compound prevents the Botulinum Neurotoxin A from working in cells, and has been shown to be well-tolerated in neuronal cell cultures at high concentrations. Optimization of this lead is underway in collaboration with the University of Pittsburgh. Data-mining studies are underway to find new leads against Botulinum Neurotoxin serotypes B, C, D, E and F.
- Discovery of the ability to intefere with the Ebola reproduction cycle by microtubule depolymerizers such as colchicine.
- Identification of critical targets shared by both proliferating cancers and biothreat agents such as anthrax, botulinum and filoviruses.

Nguyen TL, McGrath CF, Hermone AR, Burnett JC, Zaharevitz DW, Day BW, Wipf P, Hamel E, Gussio R. A common pharmacophore for a diverse set of colchicine site inhibitors using a structure-based approach. J Med Chem, Sep 22; 48(19):6107-16, 2005.

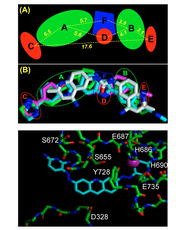
Halverson KM, Panchal RG, Nguyen TL, Gussio R, Little SF, Misakian M, Bavari, S, Kasianowicz JJ. Anthrax biosensor, protective antigen ion channel asymmetric blockade. J Biol Chem. Oct 7;280(40):34056-62, 2005.

Nguyen TL, Schoehn G, Weissenhorn W, Hermone AR, Burnett JC, Panchal RG, McGrath C, Zaharevitz DW, Aman MJ, Gussio R, Bavari S. An all-atom model of the pore-like structure of hexameric VP40 from Ebola: Structural insights into the monomer-hexamer transition. J Struct Biol. Jul; 151(1):30–40, 2005.

Burnett JC, Schmidt JJ, McGrath CF, Nguyen TL, Hermone AH, Panchal RG, Vennerstrom J, Zaharevitz DW, Gussio R, Bavari S. Conformational sampling of the botulinum neurotoxin serotype A light chain: Implications for inhibitor binding. J Bio Med Chem. Jan 17;13(2):333-41, 2005.

Structure	NSC Number	% Inhibition	K_i (μM)	Inhibition Type
	12155	95	0.8 ± 0.18	Competitive
	357756	90	4.9 ± 1.7	Competitive
	369718	90	N.D.[a]	Uncompetitive
	369721	90	4.2 ± 0.21	Competitive
	359465	48	N.D.	N.D.
	377362	33	N.D.	N.D.
	240899	0	N.D.	N.D.

[a]Not Determined



Frederick Forums Promote Scientific Interaction

Although each of the Confederation partners pursues distinct missions, they share many intersecting spheres of research. The Frederick Forums bring together scientists with common interests from across the Frederick campus to enhance intellectual interaction, collaboration, and resource sharing. Each of the Forums has a steering committee that designs communication-enhancing activities and works with the organizations' leaderships to overcome barriers impeding closer scientific interaction. Anyone interested in working with one of the Forums is encouraged to contact the Forum chair.

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Biodefense	**Bob Ulrich**, Ph.D., USAMRIID robert.ulrich@amedd.army.mil 301-619-4232
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